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THE biodesign INSTITUTE
ARIZONA STATE UNIVERSITY

Project Title: Subsurface Bio-Immobilization of Plutonium: Experiment and Model Validation Study

Principle Investigators:

Donald Reed, Earth and Environmental Sciences Division, Los Alamos National Laboratory

Bruce Rittmann, Center for Environmental Biotechnology Biodesign Institute, Arizona State University

RESEARCH OBJECTIVES

The goal of this project is to conduct a concurrent experimental and modeling study centered on the interactions of *Shewanella algae BrY* with plutonium and uranium species and phases. The most important objective of this research is to investigate the long-term stability of bio-precipitated immobilized actinide phases under changing redox conditions in biologically active systems. The long-term stability of bio-immobilized actinides (e.g. by bio-reduction) is a key criteria that defines the utility and effectiveness of a remediation/containment strategy for subsurface actinide contaminants. Plutonium, which is the focus of this project, is the key contaminant of concern at several DOE sites.

The overall hypothesis for the proposed research is that stable recalcitrant plutonium phases will prevail in biologically active systems where bio-reduction occurs. The plutonium research in progress builds on our past neptunium and uranium microbiological interaction studies. This research is divided into four Tasks:

Task 1: Bio-reduction of Pu(V) and Pu(VI) by *Shewanella algae*

Task 2: Bio-association of Pu(IV), Pu(V), and Pu(VI) with *Shewanella algae*

Task 3: Effects of microbiological activity on Bio-immobilized plutonium

Task 4: Pu phase (bio-precipitated and synthetic) stability bio-column experiments

Each of the four task identified above has a modeling and experimental component that are developed and implemented iteratively.

The benefit of this project to the biogeochemistry element of the Environmental Remediation Science Program (ERSP) is that this research and modeling study will extend current ERSP research to the bio-immobilization of plutonium. Significant contributions are expected in establishing the key plutonium-microbial interactions, the effects of redox cycling on these processes and the concurrent development of a sub-model to describe these key processes.

RESEARCH PROGRESS AND IMPLICATIONS

This three-year project is at the end of its second year. The experimental component of the project, due to its focus on plutonium, is primarily being conducted in Los Alamos labs located at the Carlsbad Environmental Monitoring and Research Center under the direction of Dr. Don Reed. There, however, are active collaborations and contributions to the experimental program from Professor Geof Smith at New Mexico State University (microbiology) who did a 1 year sabbatical on the project, Professor Tom Goyne at Valparaiso University (biochemistry of reductases) with undergraduate student involvement, and Dr. Jeff Terry at the Illinois Institute of Technology (EXAFS/XANES of environmental samples). The modeling component of the project is primarily conducted at the Center for Environmental Biotechnology Biodesign Institute located at Arizona State University by Dr. Randhir Deo and is under the direction of Professor Bruce Rittmann.

An overview of the accomplishments of this project is given by the following:

- A laboratory adapted to the special requirement of the study of microbial interactions with plutonium was established. This lab features nitrogen-atmosphere radioactive materials gloveboxes for anaerobic experiments, and a strong spectroscopic and radiochemical counting capability for the trace-level detection of actinides.
- The model CCBATCH was expanded to address the coupled effects of plutonium speciation and chemistry with biological electron donor and substrate consumption to predict the fate of plutonium in biologically-active systems.
- An extensive study of the bio-reduction of UO_2^{2+} in an Fe^{3+}-lactate system by *Shewanella algae* was completed. Results obtained confirm the enzymatic reduction pathways established by others but point to strong coupling with abiotic pathways and mechanisms that establish a key role for Fe^{2+} in the reduction of uranyl. The modeling of this system by CCBATCH was essential to quantifying and unraveling the coupled processes in this system.
- A study of the abiotic stability of Pu (VI) in the presence of dissolved iron, iron oxides, and organic complexants was completed. These results show that reduced iron species (Fe, and Fe^{2+}) are effective reducing agents for higher-valent Pu species under near-neutral subsurface anoxic conditions. Organic complexants typically found as co-contaminants reduce Pu(VI) to Pu(IV) and Pu(V).
- Significant progress was made to establish the key interactions between plutonium species and S*hewanella algae*. PuO_2^{2+} was reduced rapidly through a number of abiotic pathways in organic-rich biologically active systems. In contrast to uranium, where U(VI) is the predominant higher-valent oxidation state, these results suggest a much less important role for the Pu(VI) species and a more predominant one for Pu(V). PuO_2^{+}, which is the predominant form of Pu(V), exhibits relatively high chemical toxicity toward *S. algae* under both aerobic and anaerobic conditions and, by analogy with uranium, has strongly coupled abiotic and biotic pathways for reduction and immobilization. Modeling is in progress to help de-couple these processes.

A more detailed description of these results is given in the following sections.

Experimental Results: Uranium(VI) in the U-Fe-Lactate-*S. Algae* System

A large number of experiments were completed to establish abiotic and biotic pathways that lead to the precipitation/reduction of uranium from solution in the U-Fe-Lactate-*Shewanella algae* system. In these experiments Fe(III), introduced as an NTA complex, lactate as the electron donor, and the macronutrients phosphate and ammonium were present at milli-molar concentrations. The key result from these experiments is the predominance of abiotic pathways, even under oxic conditions, that led to the precipitation of uranyl phases. Uranium (VI) is not reduced abiotically under oxic conditions in our media. These strong abiotic pathways coupled with bio-reduction that occurred when iron (III) reduction and cell growth was evident (see Figure 1). In our system, biogenic Fe^{2+} appears to be the predominant reductant rather than direct enzymatic pathways.

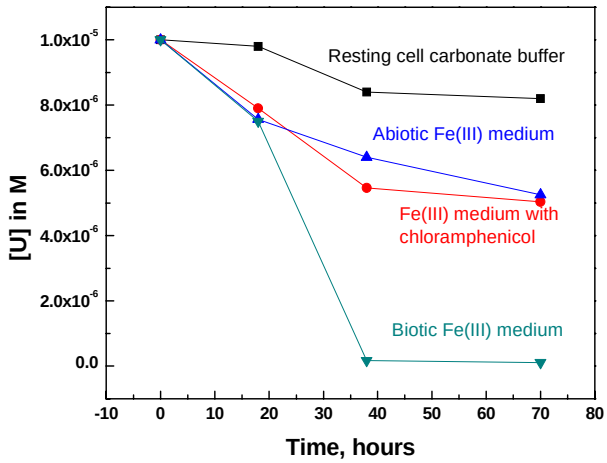

Figure 1. Bioreduction of U(VI), leading to its precipitation, only occurred when cell growth and Fe(III) reduction was observed. Choramphenicol, which is a protein synthesis inhibitor, prevented U(VI) reduction.

Experimental Results: Plutonium (V/VI) in the Pu-Fe-Lactate-*S. Algae* System

The higher valent plutonium oxidation states (di-oxo cations) are the oxidation states of most concern due to their relatively high solubility and potential for migration in the subsurface. Analogous experiments to those conducted for the uranium-*Shewanella algae* system to establish bio-reduction pathways are in progress. The presence of organic complexants and intermediates typically present in biologically active systems led to the rapid and abiotic reduction of Pu(VI) to Pu(V). For this reason, our research emphasis is focused on Pu(V).

The very low tendency of Pu(V) to form complexes (hydrolysis is not significant below pH = 8) at near-neutral pH mean that high concentration of the aquo species can be present in solution. This, as shown in Figure 2, leads to relatively high chemical toxicity towards *Shewanella algae* that is almost completely suppressed when the Pu(V) is present as an organic complex.

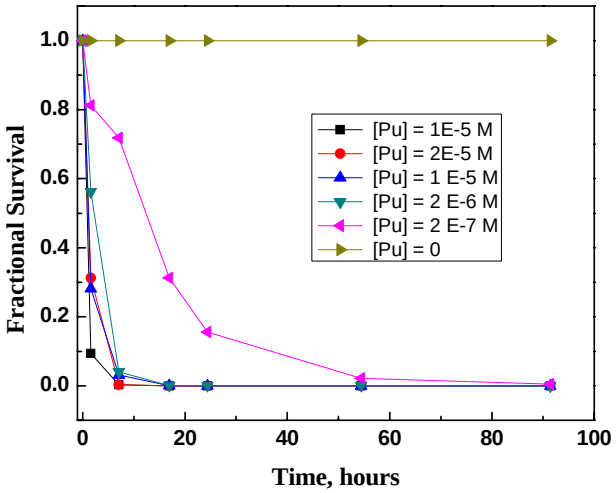

Figure 2. The toxicity of Pu(V), as the PuO2+ aquo species, towards *shewanella algae*. Even 10^{-7} M concentrations led to high toxicity. The microbe retained significant viability for weeks under the conditions of our experiments.

We have already established some of the key interactions of Pu(V) with S*hewanella algae*. The extent of bio-association of Pu(V) is very small , typically under 5% for cell densities of 10^7/mL. This is consistent with the highly non-complexing chemistry of the Pu(V) species. Under anaerobic condition, we have demonstrated that direct enzymatic reduction of Pu(V) to sparingly soluble Pu(IV) species occurs (see Figure 3). This also occurs due to reaction with biogenically produced Fe^{2+}. Strong coupling between abiotic and biotic pathways, probably more so that in the case of uranium was also observed.

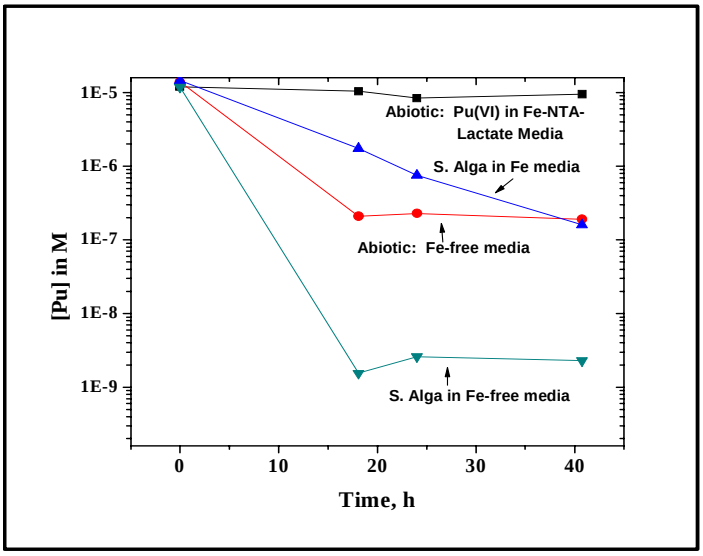

Figure 3. Bioreduction of Pu(V/VI) by *shewanella algae* in an Fe-NTA-lactate medium.

Modeling Development for the *Shewanella algae*-Pu/U system

Radionuclide bioremediation is inherently complicated, since the radionuclides participate in many different biotic and abiotic reactions that proceed at vastly different rates. Such a complex scenario can be understood and controlled only by using mathematical modeling. A unique biogeochemical mathematical model, CCBATCH, was developed by our team to connect all the different types of reactions that control the fate of radionuclides and a range of other metals and organic co-contaminants.

Modeling activities in Center for Environmental Biotechnology have centered on upgrading the CCBATCH biogeochemical model to include anaerobic growth of *S. algae* and relevant uranium and plutonium speciation data. We added new components and complexes to the model, along with their respective equilibrium constants and stoichiometric mass balances. One of the biggest modeling challenges had been to adapt the model to represent anaerobic bacterial growth that depends on reducing Fe^{3+}, not oxygen, as the primary electron acceptor, since the U and Pu reducers are Fe^{3+} reducers. The challenge is that Fe^{3+} complexes with many anionic species, and these complexes may or may not contain bio-available Fe^{3+}. Among the anions, NTA^{3-} forms dominant complexes with Fe^{3+}, with $FeOHNTA^-$ being the most dominant form at pH 7. Previously, the model assumed that all forms of Fe^{3+} are equally bio-available. We are experimenting with different ratios of Fe^{3+} and NTA to determine which Fe^{3+} species are bio-available, since the form strongly affects the kinetics of Fe^{3+} reduction. In order to elucidate the bio-available form of Fe^{3+}, we upgraded CCBATCH to allow selected complexes of Fe^{3+} as primary electron acceptors. Figure 4 shows simulated results of the effect of Fe^{3+}-NTA ratio on the production of total-Fe^{2+} when different Fe^{3+} species are the bio-available electron acceptor: (A) total-Fe^{3+} and (B) $FeOHNTA^-$. While total-Fe^{3+} produces total-Fe^{2+} at a consistent rate, the pattern is very different if $FeOHNTA^-$ is the only available species. At the higher concentration of NTA, the non-dominance of $FeOHNTA^-$ slows the production of total-Fe^{2+}. These results illustrate how we can determine the bio-available species of Fe^{3+}, as well as of U and Pu, by matching such simulated results to experimental results with different ratio of Fe^{3+}:NTA or with different pH values, which also alter the speciation.

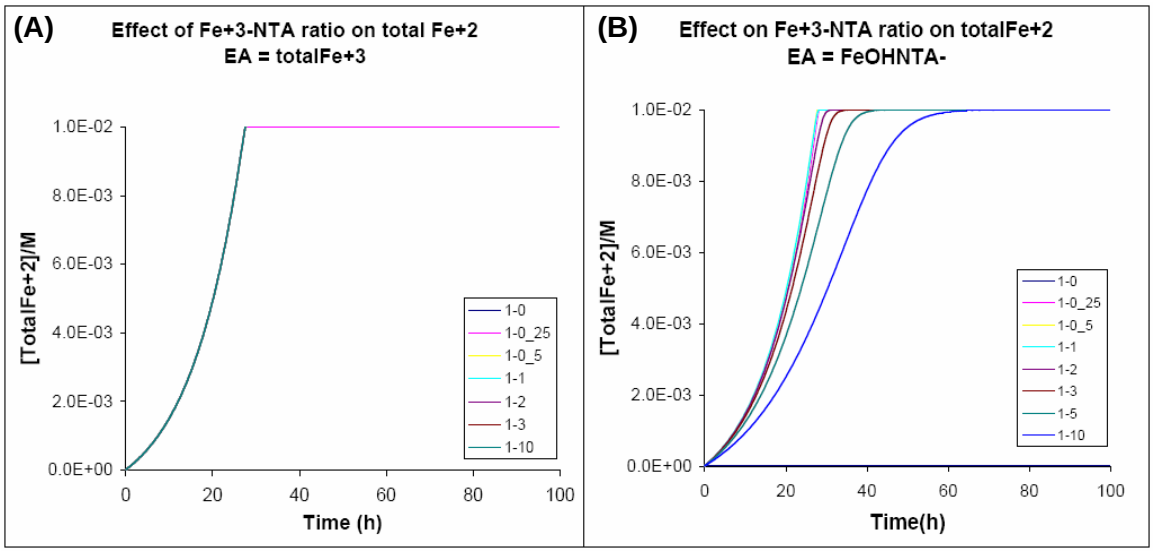

Figure 4: Simulation of the effect of Fe^{3+}:NTA ratio on the production of total Fe^{2+} when different forms of Fe^{3+} were used as electron acceptors (A) total Fe^{3+} and (B) FeOHNTA- EA indicates the chosen electron acceptor.

PLANNED ACTIVITIES

- Establish by modeling and experiment the role of Fe^{2+} in the bio-reduction of U(VI) and Pu(V/VI) by *Shewanella algae* (This is Task I, and is 90% complete)
- Synthesize a series of environmentally-relevant plutonium phases and establish the effects of redox cycling in the Shewanella algae system (This is Task III, and will be initiated by 1/07)
- Establish the reactivity of Fe^{2+} towards higher valent actinides (This supports Tasks 1 and 3 and is 30% complete)
- Complete the upgrade of CCBATCH to include Pu speciation (75% complete)

PRESENTATIONS AND PUBLICATIONS

D. T. Reed, B. E. Rittmann and W. Songkasiri, "Biosorption of Neptunium (IV) and Neptunium (V) on Soil Bacteria", presented at the Goldschmidt conference, Idaho, May 2005.

D. T. Reed, B. E. Rittmann, W. Songkasiri, and J. F. Lucchini, "Bioreduction of Plutonyl and Neptunyl by S. Algae", presented in Migration 2005, Avignon, France, September 2005.

D. T. Reed, G. Smith, R. Deo, B. Rittmann, J. F. Lucchini, M. Borkowski, M. K. Richmann, "Subsurface Bio-mediated Reduction of Higher-Valent Uranium and Plutonium" presented at Plutonium Futures, Monterey, CA, July 2006. Subsequent paper is in preparation.

W. Songkasiri, B. E. Rittmann, and D. T. Reed, "Surface Complexation of Neptunium onto Bacterial Cell Surfaces", in preparation for submittal to J. Env. Sci.

R. Deo, B. Rittmann, D. T. Reed, G. Smith, "Subsurface Bio-Immobilization of Plutonium: Experimental and Model Validation Study" presented at 19th Rocky Mountain Regional Meeting of the American Chemical Society, Tucson, AZ, October 2006.

R. Deo, B. Rittmann, D. T. Reed, G. Smith, "Subsurface Bio-Immobilization of Plutonium: Experimental and Model Validation Study" to be presented at the Pittcon2007, Chicago, March, 2007.